Fc-Receptor-Bearing Macrophages Isolated From Hypersensitivity and Foreign-Body Granulomas

Delineation of Macrophage Dynamics, Fc Receptor Density/Avidity and Specificity

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Foreign-body and delayed hypersensitivity granulomas were induced in mice; and the dynamics of macrophages isolated from dispersed, 1–4-week-old lesions was delineated. The size and histologic complexity of the lesions increased as shown: adjuvant > schistosome egg > methylated bovine serum albumin > bead. Esterase staining, spreading on glass, and the percentage of Fc-receptor-bearing macrophages present in the various granulomas reflected the same gradient. The Fc receptors were examined by rosetting with rabbit-antibody-SRBC complex (EA). Whereas more than 90% of the population of macrophages of the dermal adjuvant granuloma contained undiminished numbers of receptor-bearing macrophages throughout the 4 weeks, the percentage of macrophages that displayed receptors in pulmonary foreign-body (40%) and delayed hypersensitivity granulomas (70%) peaked at 1 week and subsequently declined. The EA rosetting of the foreign-body and delayed hypersensitivity granuloma macrophages was strongly inhibited by monomeric IgG2a-specific and weakly by aggregated IgG2b-specific mouse myeloma proteins. Also, macrophages of the delayed hypersensitivity granulomas rosetted in higher percentages with SRBCs coupled with monomeric IgC2a than with those coupled with aggregated IgG2b myeloma proteins. Macrophages of the foreign-body lesion did not react with aggregated IgG2b-SRBC. Rosetting with monomeric IgG2a-SRBC or aggregated IgG2b-SRBC could not be cross-inhibited by the myeloma proteins. Both the monomeric IgG2a-SRBC and aggregated IgG2b-SRBC complexes were readily phagocytized. Trypsin treatment of the macrophages inhibited rosetting with EA or myeloma-protein-coupled SRBCs. The display of Fc receptors on the granuloma macrophages seems to be related to the etiology of the lesion and the intensity and duration of the inflammatory reaction. (Am J Pathol 96:457–476, 1979)

GRANULOMAS are focal, chronic, predominantly mononuclear tissue inflammations evoked by persistent irritants. The composition of the irritant and its degradability and immunogenicity are decisive factors in the etiology (nonimmune, foreign body, or delayed hypersensitivity) and development of the lesions.[1] Although the bulk of both the foreign-body and delayed hypersensitivity granulomas is composed of macrophages and their derivatives, the cell populations of these lesions nevertheless differ in the rate of cellular turnover,[2] susceptibility to immunosuppressive measures,[1] antimacrophage serum,[3] and carrageenan

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treatment.[4] Recently it has been shown that inflammatory alveolar [5] and peritoneal [6-8] macrophages, monocytes from sarcoid patients, [9-10] as well as established macrophage-like lines [7-11] display increased numbers of Fc receptors on their membranes. Increased display of such receptors is also considered to reflect a stage in macrophage maturation.[12-13] In the present study we delineated the dynamics of Fc-receptor-bearing macrophages in the various granulomas. The concentration of these receptor-bearing macrophages was in correlation with the etiology and age of the lesions and the intensity of the inflammatory response.

Materials and Methods

Animals

CBA/J female mice, 22–26 g in body weight (Jackson Labs, Bar Harbor, Maine), were used in all experiments performed.

Bead Preparation and Covalent Binding of Antigen

Cyanogen-bromide-activated Sepharose 4B beads (Pharmacia, Piscataway, NJ) were sized to 40–50 μ by sieving through a Brown capsule.[14] Using a 0.2-M sodium-borate-boric-acid buffer, pH 8.2, 0.5 ml of a 2 g/ml slurry of the beads was coupled with 0.7 ml of a 30 mg/ml solution of methylated bovine serum albumin (MeBSA) (Sigma Chemical Co., St. Louis, Mo).[15] The coupling was allowed to procede for 18 hours at 4 C on a tube rotator, after which the beads were thoroughly washed and adjusted to a 14,000 bead/ml concentration with saline. The animals were given injections of 0.5 ml of this preparation. Additional activated Sepharose 4B beads were treated with 0.7 ml of a 1.0-M diethanolamine solution, which plugged the activated sites of these beads. Such naked, plugged beads were used for the generation of the foreign-body-induced granuloma.

Schistosome Egg Harvest

Schistosome eggs were harvested by the method of Coker and Lichtenberg [14] from the livers of mice infected with *Schistosoma mansoni*. The eggs were washed and resuspended in saline for injection.

Generation of Hypersensitivity-Induced and Foreign-Body-Induced Granulomas

Dermal adjuvant granulomas were produced by emulsifying equal volumes of saline and complete Freund's adjuvant (Difco Laboratories, Detroit, Mich) and injecting subcutaneously 0.2 ml of this emulsion into the neck region.

Primary MeBSA lung granulomas were produced by injecting intravenously 7000 MeBSA-coupled Sepharose beads into unsensitized mice. To produce secondary MeBSA lung granulomas, mice were sensitized subcutaneously with 200 μg MeBSA in a 0.2-ml volume of complete Freund's adjuvant emulsion. Ten days later, 7000 MeBSA-coupled Sepharose beads were injected intravenously into these mice.

Primary schistosome-egg-induced granulomas were produced by injecting intravenously 7000 *S mansoni* eggs into unsensitized mice.

Secondary schistosome-egg-induced granulomas were produced by sensitizing mice intraperitoneally with 1500 *S mansoni* eggs, followed one week later by the intravenous injection of 5000 *S mansoni* eggs.

Foreign-body granulomas were produced by the intravenous injection of 7000 diethanolamine-plugged Sepharose 4B beads into normal mice.

Footpad Tests

The state of delayed hypersensitivity in the 7-day sensitized mice was verified by a skin test. Groups of 6 animals received injections in the right hind footpad of 30 μg in 0.03 ml of MeBSA, soluble schistosome-egg antigen (SEA), or purified protein derivative (PPD). The contralateral pads received saline. Twenty-four hours later the swelling was measured by the use of a micrometer. The net swelling after subtracting the values of the control pads was expressed in millimeters. Net swelling in MeBSA-sensitized, schistosome-egg–sensitized, and complete-adjuvant–sensitized mice was 0.66, 0.62, and 0.34 mm, respectively.

Histology and Granuloma Quantitation

Histologic sections (5 μ thick) were stained with hematoxylin and eosin. Mean granuloma diameters were calculated by taking two measurements at right angles with an automated image splitter (Vickers Instruments, Woburn, Mass).

Esterase Staining

Macrophage monolayers were stained for esterase content by the method of Yam et al,[16] by the use of the substrate α-naphthyl butyrate (Sigma) at pH 6.0.

Granuloma Harvest and Dispersal

At weekly intervals, after the initiation of the granulomatous reaction, groups of animals were anesthesized and exsanguinated by ocular bleeding. The subcutaneous adjuvant granulomas were excised, cut open, rinsed to remove residual adjuvant material, and minced with scissors. To obtain pulmonary granulomas, the lungs were removed, suspended in RPMI 1640 medium, and blended for 30 seconds at low speed in a Waring blender.

Granulomas were collected on a screen and were incubated for 30 minutes at 37 C in a 10-mg/ml collagenase solution (Grand Island Biochemicals Co., Inc., Grand Island, NY) dissolved in RPMI 1640, and supplemented with 10% FCS.[17] After incubation, the treated granulomas were mashed through a wire screen and gently dispersed to obtain a single cell suspension. Cells were then allowed to adhere to glass tissue-culture slides (Lab Tek Products, Naperville, Ill) for at least two hours and then were vigorously washed to remove nonadherent cells.

Preparation of Sensitized Rabbit-Antibody-SRBC Complex (EA)

Sheep erythrocytes in Alsever's solution (Cleveland Scientific Co., Cleveland, Ohio) were washed three times in RPMI 1640. Aliquots of a 5% SRBC suspension were incubated with equal volumes of the 7S IgG fraction of rabbit anti-SRBC antibody (Cordis Laboratories, Miami, Fla) previously diluted over a range of 1:250–1:16,000. The 1:250 dilution was the dose just above the agglutinating concentration. After a 30-minute incubation at 37 C, the erthyrocytes were washed three more times and resuspended to a 0.5% concentration in RPMI 1640.

Rosette Assay

Adherent macrophage monolayers were covered with 0.5 ml of the EA suspension. Following incubation at room temperature for 20 minutes, the monolayers were gently washed with RPMI 1640. The slides were stained with Leishman's stain, and the percentage of rosetting cells having characteristic macrophage morphology was determined. Thus, lymphocytes, neutrophils, eosinophils, and occasional fibroblasts were excluded from the counts. Macrophages with three or more attached sheep erythrocytes were counted as rosetted cells. At least 200 cells were counted in each monolayer.

Phagocytosis Assay

Latex beads (0.81 μ; Difco), in a 0.5% suspension of SRBCs sensitized with a 1:250 dilution of antibody or a 1.0% suspension of SRBCs coupled with monomeric IgGa or heat-aggregated IgG2b, were incubated with adherent macrophage monolayers in RPMI 1640, supplemented with 20% FCS for one hour at 37 C. Unsensitized or uncoupled erythrocytes were not phagocytized. Cells phagocytizing latex particles were counted under phase contrast using a Zeiss standard microscope.

Trypsinization

Macrophages were added to a 200 μg/ml trypsin solution (TRTPCK, Worthington Biochemical Corp., Freehold, NJ) in a ratio of 10^7 cells per 0.5 ml of the trypsin solution.[11] The cells were incubated in the trypsin solution for 30 minutes at 37 C, washed three times in RPMI 1640, plated, and rosetted as previously described.

Rosette Inhibition With Myeloma Proteins

A 1:2 dilution of IgG2a (UPC 10), IgG2b (MOPC 195), IgA (TEPC 15), IgG1 (MOPC 21), IgG3 (FLOPC 21), and IgM (MOPC 104E) class-specific myeloma proteins (1 mg/ml protein, Bionetics, Inc., Kensington, Md) was prepared. IgG2b was first heat-aggregated at 63 C for approximately 15 minutes, until slightly opalescent. The adherent macrophage monolayers were preincubated for 15 minutes at room temperature with 0.4 ml of the monomeric or aggregated myeloma proteins, washed, and rosetted with EA. The dilution of sensitizing antibody was adjusted so that it would provide a 50% rosetting.

Direct Rosetting with E-Myeloma Proteins

The monomeric myeloma proteins, IgA, IgG3, IgG2a, and heat-aggregated IgG2b, were coupled to the sheep erythrocytes with chromic chloride, using the method of Parish and Hayward.[18] Adherent macrophages were rosetted for 20 minutes at room temperature with a 1% solution of these coupled erythrocytes.

Results

Comparison of the Size, Histology, and Macrophage Activity of the Various Granulomas

The various pulmonary granulomas were measured in stained histologic sections. Whereas the lung lesions peaked in size at 7 days and subsequently diminished, the size of the macroscopic adjuvant granulomas that encased the oily emulsion did not decrease over the 4 weeks of the study. Comparison of the diameters of the 7-day-old lesions showed a wide range, the smallest being the foreign-body bead and the largest the adjuvant-induced granuloma. The granuloma macrophages also reflected a gradual increase in the intensity of esterase staining and the ability to spread on glass. It is noteworthy that the cells of the natural, egg-induced hypersensitivity granulomas exhibited stronger staining and better spread than their counterparts, the artificial MeBSA lesions (Table 1). Histologically, the granulomas also presented a picture of increasing complexity. The naked beads were surrounded by a layer or two of immature macrophages. The bead-induced hypersensitivity granuloma was composed of

Table 1—Comparison of the Size and Macrophage Activity of the Various Granulomas

Type of Granuloma*	Diameter ($\mu \pm$ SE)	Macrophage activation	
		Esterase staining	Spreading on glass
Foreign body	63.5 ± 1.1	+	±
Secondary MeBSA	148.1 ± 6.0	++	+
Secondary schistosome egg	268.3 ± 9.9	+++	+++
Adjuvant	>1000	++++	++++

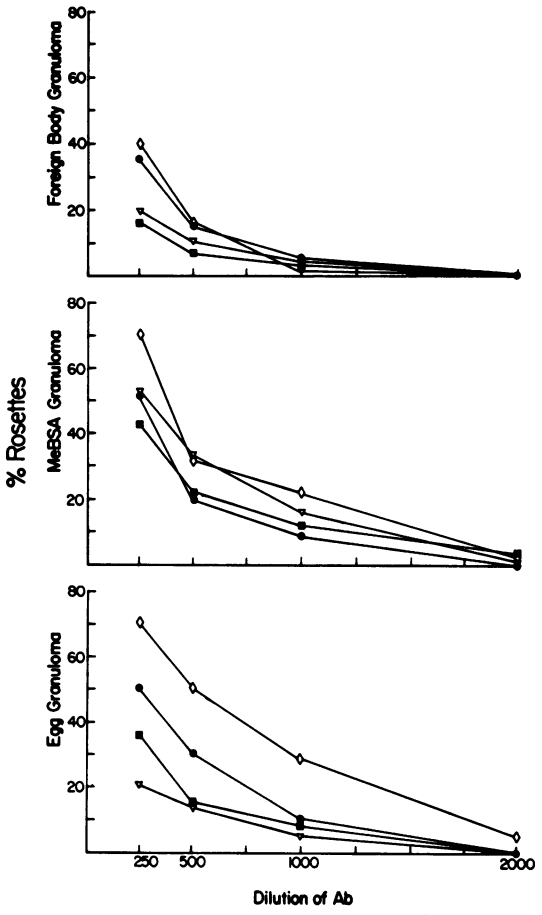
* Values from 7-day-old lesions

lymphocytes, enlarged macrophages, few epithelioid cells, and occasional giant cells. The schistosome-egg lesion had a similar composition, with the addition of considerable numbers of eosinophils. Finally, the subcutaneous adjuvant granuloma showed the greatest complexity. Inside the fibrous capsule the vascularized lesion contained fibroblasts, many large microphages, polymorphonuclear cells, typical epithelioid cells, and lymphocytes (Figure 1).

Enumeration of the Fc-Receptor-Bearing Macrophages From Dispersed Foreign-Body and Hypersensitivity Granulomas

Since the granuloma macrophages were obtained following the dispersal of the lesions by collagenase, the effect of this enzyme on the Fc receptors of macrophages was examined. Macrophage monolayers obtained after enzymatic dispersion of the granulomas were rosetted immediately and after overnight incubation in medium containing 10% FCS. No increase in the number of rosetting cells was seen. In addition, thioglycollate-induced (3-day) peritoneal exudate macrophages were incubated in RPMI 1640 with collagenase to simulate the conditions of the granuloma dispersal, and then were rosetted with EA. Since the percentages of rosetting macrophages before (51.3%) and after (51.0%) enzyme treatment were unchanged, we concluded that the receptors were undamaged by enzyme treatment.

The granuloma macrophages obtained from the 1–4-week-old lesions were allowed to rosette with erythrocytes sensitized with decreasing concentrations of rabbit IgG antibodies. The ability of macrophages to rosette with lightly sensitized cells is considered to be a measure of the density/avidity of the Fc receptors displayed on the cell membranes.[6,13] As one can see in Text-figure 1A, only about 40% of the macrophages obtained from the 7-day-old foreign-body granuloma rosetted with heavily sensitized erythrocytes. This number decreased to 17% by 4 weeks. About 5% of the



TEXT-FIGURE 1—EA rosette formation by macrophages isolated from hypersensitivity and foreign-body lung granulomas. Macrophages were isolated from 1-week (◇), 2-week (●), 3-week (▽) and 4-week (■) granulomas. Abscissa indicates dilutions (1:250–1:4000) of 7S rabbit IgG anti-SRBC used for sensitization. Each point represents three separate experiments performed on at least 9 mice.

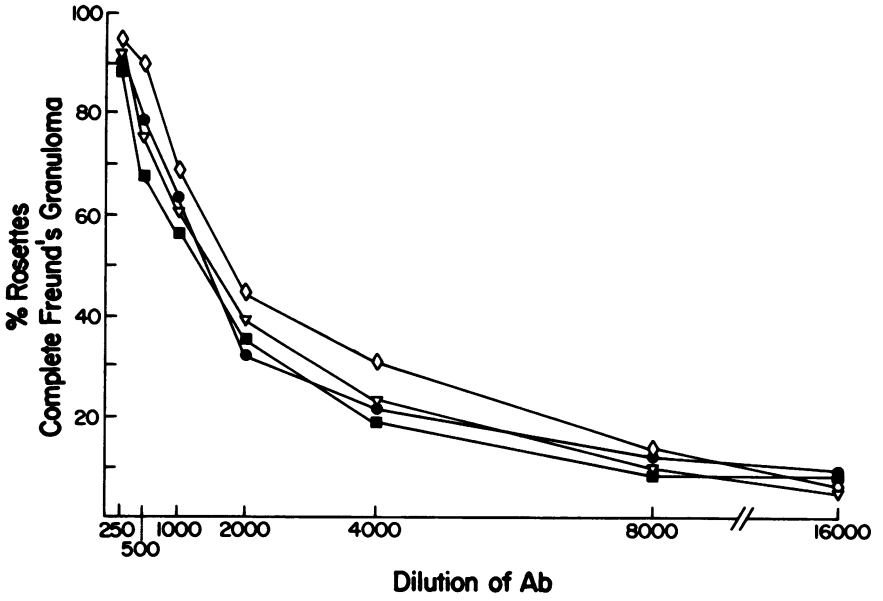
cells rosetted with lightly (1:1000 dilution of antibody) sensitized erythrocytes.

At 1 week, the secondary MeBSA-bead granuloma yielded a significantly higher percentage of rosetting macrophages (70%), which gradually decreased to 43% in the 4-week-old lesion (Text-figure 1B). Furthermore, the macrophage population of the 1-week-old lesions was more heterogeneous, in that it contained about 25% of cells that rosetted with lightly sensitized erythrocytes. These cells, displaying high density/avidity receptors, also decreased in number with the age of the lesion.

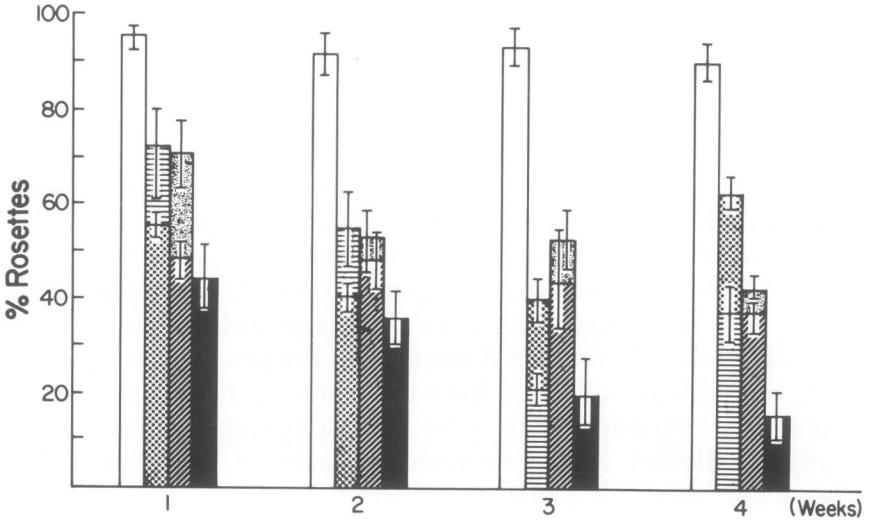
In the dynamics of the macrophages, the secondary schistosome-egg–induced granuloma was different from its artificial counterpart (Text-figure 1C). Whereas at 1 week 70% of the cells rosetted, over the next 2 weeks this number decreased sharply, only to rise again in the 4-week-old

lesion. It is noteworthy that at 1 week the number of cells displaying high density/avidity receptors was similar to that of the MeBSA lesion (30%).

The adjuvant granuloma yielded the highest percentage of receptor-bearing macrophages (Text-figure 2). Practically all the macrophages rosetted with sensitized erythrocytes, and their numbers remained unchanged over the 4-week period. The percentage of cells that reacted with lightly sensitized erythrocytes (1:1000 dilution of antibody) was remarkably high (70%); furthermore, a low number (6–13%) of macrophages also rosetted with erythrocytes sensitized with extremely diluted (1:8000–16,000) antibodies. Text-figure 3 summarizes the percentages of receptor-bearing macrophages obtained from the various granulomas. The graph also presents the number of receptor-bearing macrophages isolated from the primary MeBSA and schistosome-egg–induced granulomas. In the former, a low but steady population of receptor-bearing macrophages was evident that changed little over the 4-week period. However, the delayed hypersensitive nature of these lesions could not be verified, since the mice remained skin-test–negative throughout the period of the study. In contrast, macrophages of the primary schistosome-egg granulomas showed an actual increase in the number of receptor-bearing cells in the 4-week-old



TEXT-FIGURE 2—EA rosette formation by macrophages isolated from subcutaneous FCA granulomas. Macrophages were isolated from 1-week (◇), 2-week (●), 3-week (▽) and 4-week (■) granulomas. Abscissa indicates dilutions (1:250–1:16000) of 7S rabbit IgG anti-SRBC used for sensitization. Each point represents three separate experiments performed on at least 9 mice.



TEXT-FIGURE 3—Comparisons of EA rosette formation between hypersensitivity and foreign-body granulomas. Macrophages isolated from FCA granulomas (□), secondary MeBSA granulomas (▤), primary MeBSA granulomas (▩), secondary schistosome-egg granulomas (▦), primary schistosome-egg granulomas (▨), and foreign-body granulomas (■) were rosetted with SRBCs sensitized with a 1:250 dilution of 7S rabbit IgG anti-SRBC. Percentage of EA rosettes ± SE was determined at 1, 2, 3, and 4 weeks.

lesions. Such lesions are of a delayed hypersensitive nature, since parasite eggs can induce systemic sensitization via the intrapulmonary route.[19] Addressing the question of the identity of the adherent, mononuclear nonrosetting cells, three alternative explanations were considered: 1) the cells are fibroblasts, rather than macrophages; 2) the cells are macrophages with masked or blocked receptors; 3) the cells are macrophages devoid of receptors or with very few receptors. Indirect evidence seems to rule out the first possibility. In repeated attempts granuloma fibroblasts following enzymatic dispersal did not adhere to plastic or glass surfaces within the 2 hours of the experiment. Furthermore, monolayers of CBA/J pulmonary fibroblasts did not rosette with sensitized erythrocytes. The possibility of the masked receptors was eliminated, since repeated overnight incubations of the monolayers did not increase the percentage of rosetting cells. The third alternative seemed plausible, since the percentage of the rosetting cells of the foreign-body granuloma increased from 40% to 60% when the erythrocytes were sensitized with a slightly agglutinating dose (1:200 dilution) of antibody.

Phagocytosis Assay of the Granuloma Macrophages

The percentage of rosette-forming macrophages was routinely calculated by examining adherent monolayers stained by Leishman's stain and

counting cells with characteristic macrophage morphology. This was necessary in order to exclude lymphocytes, neutrophils, and eosinophils from the percentage of the total count. An attempt was then made to characterize the phagocytic capacity of the rosetting cells and the nonrosetting mononuclear cells. To this end monolayers obtained from the various 7-day-old granulomas were allowed to rosette at room temperature and were then incubated at 37 C for 1 hour. Monolayers were vigorously washed with buffer to remove noningested erythrocytes, and the macrophages that contained ingested erythrocytes were counted. Slides were also fixed and stained, and the ingestion of erythrocytes was verified under oil immersion. In the foreign-body granuloma the percentage of macrophages with ingested erythrocytes was 48.4. The percentage of phagocytosis in both the secondary MeBSA- and egg-induced granuloma populations was 68.0 and in the adjuvant granuloma cells 100. Thus, essentially, all those macrophages that rosetted with EA also were capable of ingesting the sensitized erythrocytes. In order to delineate further the status of the nonrosetting mononuclear cells, monolayers obtained from 7-day-old foreign-body and secondary schistosome-egg granulomas were fed latex particles and incubated at 37 C for 1 hour. Afterwards, monolayers were examined under phase contrast on the microscope. All the nonrosetting mononuclear cells from both types of lesions exhibited phagocytosis of latex particles.

Inhibition of EA Rosetting by Mouse Myeloma Proteins

Attempts were made to inhibit EA rosetting by preincubating the monolayers with monomeric mouse myeloma proteins specific for IgM, IgA, IgG1, IgG3, IgG2a, and IgG2b. With the exception of IgG2a myeloma protein, no inhibition in the EA rosetting was observed. As seen in Table 2, regardless of the type of the granuloma, the rosetting of the macrophages was inhibited by about 70% by monomeric IgG2a and only by 10–20% with heat-aggregated IgG2b myeloma proteins.

Rosetting of Macrophages With Myeloma Proteins Coupled to Sheep Erythrocytes

To further confirm that the granuloma macrophages possessed Fc receptors with IgG subclass specificity, we coupled monomeric IgA, IgG3, IgG2a, and aggregated IgG2b myeloma proteins onto sheep erythrocytes and carried out the rosette assay. No rosetting was observed with IgA- and IgG3-coupled erythrocytes. As Table 3 illustrates, the macrophages of the foreign-body granuloma rosetted weakly with IgG2a-coupled erythrocytes and essentially did not react with IgG2b-coupled erythrocytes. The hypersensitivity bead and schistosome-egg granulomas showed a moderate rosetting with the IgG2a and low rosetting with IgG2b-coupled erythro-

Table 2—Inhibition of EA Rosetting by Preincubation with Mouse IgG2a and IgG2b* Myeloma Proteins

Type of granuloma†		Percentage of inhibition after preincubation with IgG2a	IgG2b
Foreign body		76.0	10.2
Primary	MeBSA	68.8	14.5
Secondary	MeBSA	72.9	21.7
Primary	Schistosome egg	69.0	11.0
Secondary	Schistosome egg	73.3	7.9
Adjuvant		70.1	16.4

* Heat-aggregated IgG2b
† 7-day-old lesions

cytes. In comparison, the macrophages of the adjuvant granuloma rosetted strongly with both IgG2a- and IgG2b-coupled erythrocytes. It is noteworthy that the percentage of the macrophages that rosetted with IgG2a-coupled erythrocytes was double the percentage of macrophages that rosetted with IgG2b-coupled erythrocytes.

Phagocytosis of Sheep Erythrocytes Coupled With Myeloma Proteins

In order to define the role of the IgG2a and IgG2b receptors, a phagocytosis assay was carried out on monolayers obtained from secondary schistosome-egg and adjuvant granulomas. Monolayers were allowed to rosette with erythrocytes coupled either with monomeric IgG2a or aggregated IgG2b myeloma proteins and then were incubated at 37 C for 1 hour. The percentage of phagocytic cells was counted in vigorously washed slides as described above. As Table 4 shows, there was a close correspondence between the rosetting of the macrophages and their ability to ingest the myeloma-protein–coupled erythrocytes.

Table 3—Direct Rosetting of Macrophages with SRBCs Coupled With Mouse IgG2a and IgG2b* Myeloma Proteins

Type of granuloma†		Percentage of Rosettes ± SE E-IgG2a	E-IgG2b
Foreign body		10.6 ± 2.0	1.7 ± 1.0
Primary	MeBSA	31.8 ± 0.7	3.2 ± 0.4
Secondary	MeBSA	30.4 ± 3.7	3.7 ± 1.0
Primary	Schistosome egg	35.7 ± 5.3	10.8 ± 0.8
Secondary	Schistosome egg	29.3 ± 3.2	5.5 ± 2.3
Adjuvant		69.0 ± 6.9	31.3 ± 3.7

* Heat-aggregated IgG2b
† 7-day-old lesions

Table 4—Relationship of Direct Rosetting and Phagocytosis of SRBCs Coupled With Mouse IgG2a and IgG2b* Myeloma Proteins

	Percentage ± SE			
	E-IgG2a		E-IgG2b	
Type of granuloma†	Rosettes	phagocytosis	Rosettes	phagocytosis
Secondary schistosome egg	41.1 ± 6.4	45.6 ± 4.5	5.6 ± 1.6	8.2 ± 0.7
Adjuvant	62.5 ± 1.7	57.9 ± 2.9	31.3 ± 3.7	38.1 ± 2.0

* Heat-aggregated IgG2b
† 7-day-old lesions

Cross-Inhibition of Rosetting by Myeloma Proteins

To determine whether separate Fc receptors existed for the IgG2a and IgG2b myeloma proteins, cross-inhibition experiments were performed with the use of the adjuvant granuloma macrophages that showed previously the strongest rosetting with the myeloma-protein–coupled erythrocytes. As one can see, whereas IgG2a and IgG2b inhibited subsequent rosetting with erythrocytes coupled with the homologous myeloma proteins, no cross-inhibition was found between the two subclasses (Table 5).

Trypsin Sensitivity of the Fc Receptors

Pretreatment of monolayers of the various granuloma (foreign-body as well as hypersensitivity) macrophages with trypsin markedly inhibited rosetting with EA (75% inhibition) or IgG2a-myeloma-protein–coupled erythrocytes (80% inhibition).

Discussion

In the present study, foreign-body and various hypersensitivity granulomas were generated. The size and histology of the lesions, the dynam-

Table 5—Cross-Inhibition of Direct Rosetting* with SRBCs Coupled With Mouse IgG2a and IgG2b† Myeloma Proteins

	Percentage of Rosettes ± SE	
Preincubation	E-IgG2a	E-IgG2b
Medium‡	62.5 ± 1.7	22.2 ± 2.6
IgG2a monomer	20.0 ± 3.8	22.6 ± 4.7
IgG2b aggregates	58.6 ± 2.4	12.7 ± 0.7

* Macrophages of 7-day-old adjuvant granulomas
† Heat-aggregated IgG2b
‡ RPMI 1640

ics of Fc-receptor–bearing macrophages, as well as the density/avidity and specificity of these receptors were compared. Whereas the foreign-body lesions were small and contained mostly undifferentiated macrophages, the larger hypersensitivity granulomas consisted of mature macrophages, epithelioid and giant cells, neutrophils, and eosinophils. In this group, graded tissue responses were seen, ranging from the MeBSA-bead–, through the schistosome-egg–, to the adjuvant-induced lesions. The intensity of the cellular reaction correlated with the quality of the antigens (bland serum protein, helminthic, bacterial) and their mode of presentation (covalently bound, soluble diffusing, particulate emulsion incorporated). The macrophages of the hypersensitivity lesions exhibited high esterase content [20] and pronounced spreading,[21] whereas the cells of the foreign-body bead granuloma showed the opposite picture.

The dynamics of the Fc-receptor–bearing granuloma macrophages was consistent with the above picture. The peak-sized, 7-day-old foreign-body granuloma yielded a heterogeneous mononuclear population. Less than half of these cells displayed receptors, and the percentage of macrophages bearing high density/avidity receptors was low. Thus most of the cells were devoid of receptors, or the sparse receptors were undetectable by rosetting. Such sparse receptors should be detectable by labeled polymerized IgG myeloma proteins.[22] As the lesion waned, the percentage of receptor-bearing macrophages markedly decreased. In contrast, the number of macrophages with receptors in the 7-day-old hypersensitivity granulomas was higher, the percentage of cells displaying high density/avidity receptors markedly elevated, and the tempo of disappearance of the receptor-bearing cells from the aging lesions was slower. Interestingly, the nonrosetting mononuclear cells of the bead-induced and schistosome-egg–induced granulomas nevertheless ingested latex particles. Although we cannot exclude the possibility that such cells are fibroblasts, we are inclined to identify these phagocytic, adherent mononuclear cells as macrophages that display nonspecific receptors for particle phagocytosis [23,24] that are independent of the Fc receptors. Similar conclusions were obtained from studies using coverslip-induced granulomas. Macrophages from the waning lesions showed diminished Fc-receptor–mediated rosetting and ingestion of EA, whereas the Fc-receptor–independent ingestion of glutaraldehyde-treated erythrocytes remained unchanged.[25]

The present data strongly suggest a correlation between lesion size, macrophage kinetics, the extent of display of Fc receptors, and the intensity and duration of the inflammation. The inflammatory signals that initiate and maintain the granulomatous response are generated after the interaction of the irritant/antigen with serum and tissue components and

continue throughout the duration of the immune response.[1] Since in all types of granulomas the number of receptor-bearing cells peaked early (7 days), the signals which prevailed during the transition of blood-borne precursor monocytes to tissue macrophages must have induced maximum display of membrane receptors.[26,27] Although as yet uncharacterized, the foreign-body granuloma fits the category of the low-turnover lesion,[2] where blood monocytes enter the area of irritation and develop into a nondividing, long-lived macrophage population. Plasma-derived kinins,[28] prostaglandins, and SRS-A [29] have been shown to participate in the generation of such lesions. Presumably, such acute, perhaps weaker signals may not induce cell division and/or the enhanced display of Fc receptors of macrophages. In contrast, the high macrophage turnover of the BCG-induced granuloma [30] is possibly due to lymphokine-mediated cell recruitment and division. Since lymphokine production by the schistosome egg [31,32] and MeBSA granulomas has been demonstrated (manuscript in preparation), it is likely that the hypersensitivity-type egg [33] and artificial granulomas [34-36] also have a lymphokine-mediated high macrophage turnover. Lymphokines were shown to affect macrophage membranes, inducing spreading, membrane ruffling,[37] differentiation,[38] immobilization by aggregation,[39] and fusion.[40,41] Thus, the enhanced display of Fc receptors would be an additional manifestation of increased membrane activity. Indeed, increased numbers of Fc receptors appear on macrophage membranes during maturation [6,12,13] and after inflammatory stimulation of the peritoneum [6-8] and lungs.[5] More importantly, increased display of Fc receptors on circulating blood monocytes from patients with sarcoidosis, Crohn's disease, and tuberculosis has been demonstrated.[9,10]

The Fc receptors of the granuloma macrophages were further characterized with respect to specificity, trypsin sensitivity, and mediation of phagocytosis. Using mouse myeloma proteins of various classes and subclasses, we showed that macrophages of the foreign-body and hypersensitivity lesions have receptor specificity only for the IgG2a and IgG2b subclasses of immunoglobulins. The lowered percentage of rosetting seen in these experiments is attributed to the less favorable orientation of the Fc portions of myeloma proteins on the erythrocytic surface and the diminished capacity of the receptors to bind aggregated IgG2b myeloma proteins of unknown size.[22] It is noteworthy that in hypersensitivity lesions most cells showed IgG2a, whereas only a minority displayed IgG2b-specific receptors. In comparison, the percentage of macrophages from foreign-body lesions with IgG2a-specific receptors was lower, and those with IgG2b-specific receptors were negligible. Since the EA rosetting was markedly inhibited by monomeric IgG2a-specific myeloma proteins and

weakly by IgG2b-specific myeloma proteins, we concluded that rabbit IgG bound predominantly to IgG2a-specific receptors and to a lesser extent to IgG2b-specific receptors. Cross-inhibition studies conducted with the macrophages of the most reactive adjuvant granuloma have conclusively shown that the cells displayed two distinct receptors: one for the IgG2a and another for the IgG2b subclass of immunoglobulins. Thus the predominantly IgG2a-specific receptor activity of the granuloma macrophage does not differ from that of the elicited peritoneal macrophages [6,7] and established macrophage lines.[7,11,27,42,43] Similarly, trypsin treatment of macrophages of the nonimmune and immune lesions abolished rosetting with EA and erythrocytes coupled with IgG2a- and IgG2b-specific myeloma proteins. This result is in accord with reports that demonstrated trypsin sensitivity of IgG2a-specific Fc receptors on elicited macrophages and established macrophage lines.[7,27,42-45] In contrast, some reports indicated that receptors that engaged aggregated or polymeric IgG2b [22,43] or immune complexes [45-49] were trypsin-resistant. The conflicting results may well be due to methodologic and species differences.

The functional significance of the two separate receptors on the macrophage membrane is still not clear. Macrophages not only rosetted with, but also ingested, the erythrocytes coupled with IgG2a or IgG2b myeloma proteins, indicating that both receptors mediated phagocytosis. Since IgG2a-specific receptors may bind cytophilic antibodies, whereas receptors for IgG2b may accommodate antigen–antibody complexes,[11] the presence of a smaller subpopulation of granuloma macrophages with IgG2b-specific receptors reactive with complexes is of major interest. Such cells present in the immune granuloma may bind, ingest, and degrade complexes and thus may affect the duration [50] and pathologic characteristics of the granulomatous inflammation by influencing macrophage migration,[51] release of lysosomal enzymes,[52] and neutral proteases.[53]

In summary, the present data revealed marked differences in cell kinetics and the display and specificity of Fc receptors of macrophages obtained from the various granulomas. These differences are related to the etiology of the granuloma and the intensity and duration of the inflammatory signals that prevail in the lesions. Further studies are needed to make clear the roles of the macrophages with different receptor specificities in determining the immunopathology of granulomatous inflammations.

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[Illustrations follow]

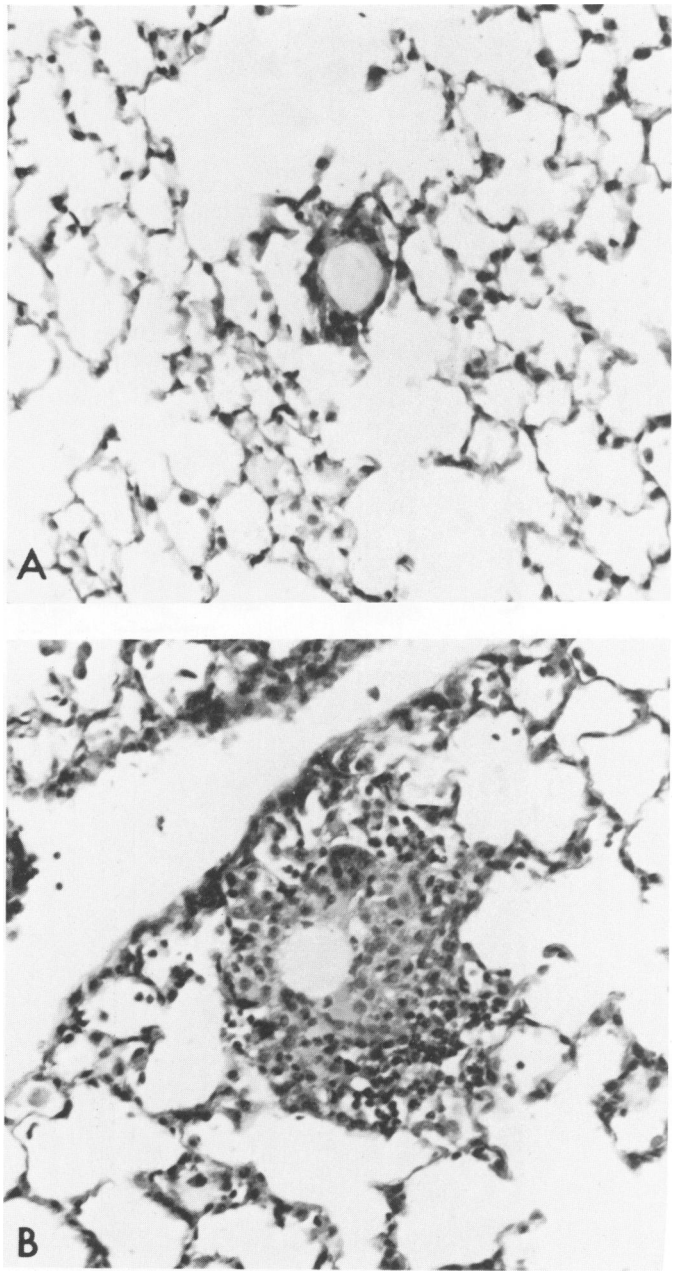
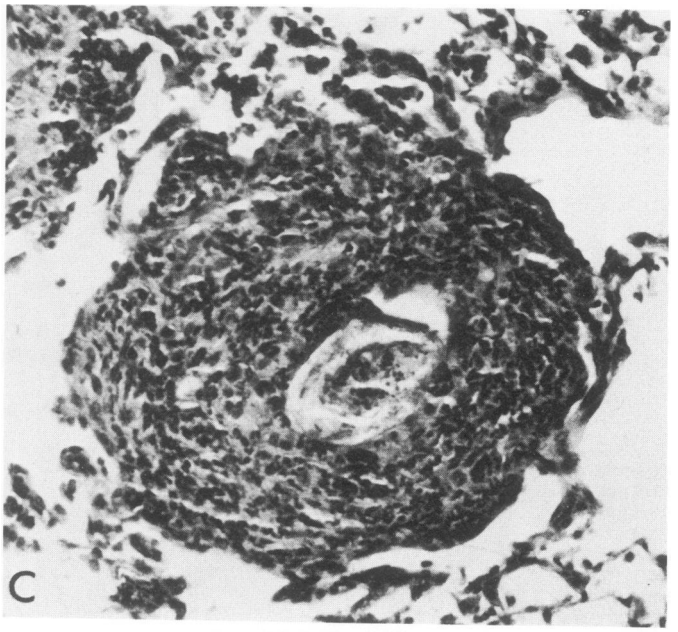


Figure 1A-C—Representative pulmonary granulomas (mean granuloma diameter determined; sections searched for granuloma representative of mean; granuloma marked and photographed). **A**—A 7-day-old foreign body granuloma. **B**—A 7-day-old secondary MeBSA granuloma. Note bead (*white circle*) in center of lesions. **C**—A 7-day-old secondary schistosome-egg granuloma. Note worm egg in center of lession. **D**—A 7-day-old dermal FCA granuloma. (H&E, ×375)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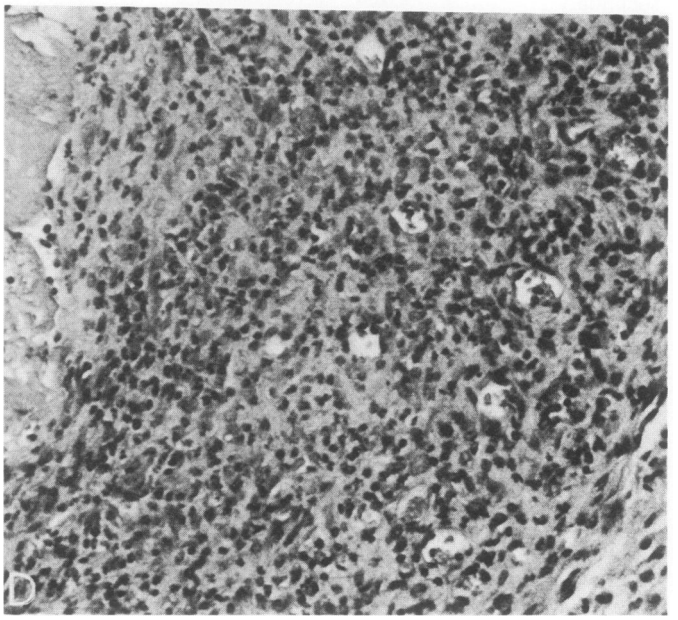
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